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Path and Association Analysis and Stress Indices for Salinity Tolerance Traits in Promising Rice (*Oryza sativa* L.) Genotypes

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Effects of salinity on correlation, path and stress indices, yield and its components were studied in a set of 34 promising rice genotypes collected from various national and international organizations. These genotypes were evaluated in a randomized complete block design with three replications during the wet seasons (*kharif*) of 2009 and 2010 in normal (EC_{iw} ~ 1.2 dS/m) and salinity stress (EC_{iw} ~ 10 dS/m) environments in micro plots at Central Soil Salinity Research Institute (CSSRI), Karnal, India. Grain yield per plant showed positive significant association with plant height, total tillers, productive tillers, panicle length, and biological yield per plant and harvest index under normal environment, whereas grain yield showed positive significant association with biological yield and harvest index under salinity stress. These results clearly indicate that selection of high yielding genotypes would be entirely different under normal and saline environments. The stress susceptibility index (SSI) values for grain yield ranged from 0.35 (HKR 127) to 1.55 (TR-2000-008), whereas the stress tolerance index (STI) values for grain yield ranged from 0.07 (PR 118) to 1.09 (HKR 120). The genotypes HKR 120, HKR 47 and CSR-RIL-197 exhibited higher values of stress tolerance index (STI) in salinity. Under salinity, negative and significant association was shown by SSI and grain yield in contrast to positive and significant association shown by STI and grain yield. These associations could be useful in identifying salt tolerant and sensitive high yielding genotypes. The stress susceptible and stress tolerance indices suggest that the genotypes developed for salinity tolerance could exhibit higher tolerance, adaptability and suitability. Harvest index and biological yield traits emerged as the ideal traits for improvement through selection and could be used to increase the rice productivity under saline stress environments.

Keywords: rice, salinity, correlation, path coefficient, SSI and STI

Introduction

Rice is the staple food and most important cereal crop which contributes more to human food requirements annually and accounts for about 43 per cent of total food grain production in India. At the current rate of population growth (1.8 per cent), rice requirement by 2020, would be around 125 million tons (Mishra 2005). The need and importance of rice is

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increasing day by day due to the increase in global population. Therefore, improving the productivity of rice is crucial for food security and economic development. Higher population growth rate and the conversion of some highly productive rice cultivation lands for industrial and residential purposes, has pushed rice cultivation to less productive area such as saline, drought and flood prone areas. In India, salt affected area accounts for 6.73 million hectares of land. Though most of the cultivated rice varieties are susceptible to salinity but variability for salt tolerance traits has also been reported (Sharma 1986, 2010; Flowers et al. 2000). The detrimental effects of salt stress are through alterations in plant metabolism, reduced water potential, ion imbalances, toxicity and reduction of crop yield and severe salt stress may lead to total failure of crop. The effects of salinity are also related to the stage of plant development at which salinity occurs, concentration and nature of the salt, and the duration of salinization (Pearson et al. 1966; Aslam et al. 1993; Zeng et al. 2001). In rice, plant height, total number of tillers, panicle length, grain weight per panicle, 1000-seed weight and quality and quantity of grains decreased progressively with increase in salinity. Involvement of some undesirable characters in the traditional varieties and complex nature of salinity tolerance have presented challenges in efforts to improve salt tolerance of crops (Yamaguchi and Blumwald 2005). Breeding of saline tolerant varieties necessitates understanding of salinity, its effects on various plant characters and including selection for one or several characters in artificially generated populations or natural populations (Simmonds 1983; Arshadullah et al. 2011). Success of any plant breeding programme depends on the magnitude of variation for yield and yield components present in the germplasm and the nature of association among these components. To improve grain yield, evaluation of germplasm is the most important aspect. Being a complex trait, grain yield is influenced by various genetic and environmental factors; therefore information on the extent of association between traits is important and should be given importance in selection. With these points in view, the present investigation was carried out to understand the effects of salinity on genetic variability, correlation, direct and indirect influences and stress indices of grain yield with important yield components in rice.

Materials and Methods

Plant materials

The experimental materials comprised of 34 promising rice genotypes collected from various national and international organizations (Table S1*). Genotypes included popular high yielding varieties, high yielding salt tolerant varieties in India and recombinant inbred lines (derived from salt tolerant and salt sensitive parents) developed by CSSRI, Karnal and elite salt tolerant breeding lines developed by IRRI, Philippines. These genotypes were evaluated in randomized complete block design with three replications during *kharif* 2009 and *kharif* 2010 in control ($EC_{iw} \sim 1.2$ dS/m) and salinity ($EC_{iw} \sim 10$ dS/m) environments in micro plots at Central Soil Salinity Research Institute, Karnal, Haryana, In-

* Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

dia, situated at 29.43º N latitude and 76.58º N longitude and 245 m above sea level. These micro plots were covered with polycarbonate sheet sheets to protect against rains. Thirty-five days old seedlings from the wet bed nurseries were transplanted two seedlings per hill with a spacing of 15 × 20 cm. Basal fertilizers for the main crop were 120–60–60 kg of NPK/ha and the recommended package of practices was followed to grow a healthy crop. One month after transplanting, salinity stress was imposed by irrigating with saline water created using 7 NaCl: 1 Na_2SO_4: 2 $CaCl_2$ salts on equivalent basis. Subsequently, salinity was recorded weekly and maintained around ($EC_{iw} \sim 10$ dSm^{-1}) throughout the cropping season.

Evaluation for yield and its yield components and stress indices analysis

Five plants from each genotype were randomly tagged in each replication and data recorded for plant height, total tillers per plant, number of productive tillers per plant, panicle length, grain yield per plant, biological yield per plant and harvest index (%). The stress susceptibility indices (SSIs) and stress tolerance indices (STIs) for each genotype were calculated for grain yield as per Fischer and Maurer (1978) and Fernandez (1993).

Statistical analysis

Homogeneity of error variance across the two seasons was tested by the F test (Gomez and Gomez 1983) and the combined analyses of variances for genotypes were performed. Significant levels were determined (McIntosh 1983) for the combined analysis. Analysis of variance (ANOVA) for all the traits was estimated (Panse and Sukhatme 1967). The genotypic and phenotypic correlations were estimated (Al-Jibouri 1958) and the genotypic correlation coefficients were partitioned into direct and indirect effects (Dewey and Lu 1959).

Results

Analysis of variance

Homogeneity of error variance across the two seasons was tested by the F test and none of the error mean squares were found significant for any of the traits. Therefore, combined analyses of variances for genotypes between the two seasons were determined by comparing the genotypes × seasons interaction for each trait. Significant levels were also determined for combined analysis. As significant differences among genotypes, seasons interaction were not observed (Table S2), data of both the seasons were therefore combined for further analysis. ANOVA revealed highly significant variation among the treatments for all the seven traits in both the control and saline environments thus suggesting the presence of sufficient variation among the genotypes along with ample scope for improving salinity tolerance in rice.

Effect of salinity on grain yield and yield attributing traits

Grain yield per plant was the trait most affected by salinity as expressed by 58 per cent reduction in saline environment compared to control non-saline environment (Table S3) and was followed by reductions in biological yield (52%), productive tillers per plant (28%), plant height (26%), total tillers per plant (24%), panicle length (18%) and harvest index (14%), respectively.

Correlation and path analysis

Grain yield per plant showed significant and positive association with plant height (0.48 and 0.60), total tillers per plant (0.26 and 0.18), productive tillers per plant (0.25 and 0.20), panicle length (0.66 and 0.71), biological yield per plant (0.68 and 0.75) and harvest index (0.85 and 0.65) at genotypic and phenotypic levels, respectively under normal environment (Table 1). The direct effects of plant height and panicle length were positive but lower in magnitude. Direct positive effects of these traits on grain yield indicated their im-

Table 1. Genotypic (G) and phenotypic (P) correlations for yield and yield contributing traits in rice in control (EC_{iw} ~ 1.2 dS/m) and saline (EC_{iw} ~ 10 dS/m) environments

Characters		Environments	Total tillers	Productive tillers	Panicle length	Biological yield	Harvest index	Grain yield
Plant height (cm)	G	Normal	0.68**	0.64**	0.66**	0.64**	0.25**	0.48**
		Saline	–0.09	0.14*	0.37**	0.35**	0.03	0.25**
	P	Normal	0.51**	0.51**	0.70**	0.71**	0.19*	0.60**
		Saline	–0.15*	–0.14*	0.38**	0.07	–0.1	–0.01
Total tillers per plant	G	Normal	1	0.96**	0.46**	0.47**	0.17*	0.26**
		Saline	1	0.49**	0.07	0.26**	0.05	0.14*
	P	Normal	1	0.96**	0.43**	0.35**	–0.01	0.18**
		Saline	1	0.73**	–0.01	0.12	0.02	0.08
Productive tillers per plant	G	Normal		1	0.46**	0.46**	0.18*	0.25**
		Saline		1	0.16*	0.022	0.20**	0.07
	P	Normal		1	0.45**	0.37**	0.001	0.20**
		Saline		1	–0.01	0.027	0.15*	0.1
Panicle length (cm)	G	Normal			1	0.24**	0.77**	0.66**
		Saline			1	–0.11	0.40**	0.16*
	P	Normal			1	0.53**	0.47**	0.71**
		Saline			1	–0.063	0.20**	0.11
Biological yield per plant (g)	G	Normal				1	0.24**	0.68**
		Saline				1	0.04**	0.76**
	P	Normal				1	0.03	0.75**
		Saline				1	0.18**	0.75**
Harvest index (%)	G	Normal					1	0.85**
		Saline					1	0.67**
	P	Normal					1	0.65**
		Saline					1	0.77**

* significant at $p = 0.05$; ** significant at $p = 0.01$

portance in determining the complex character and, therefore, should be given priority while making selections for the improving grain yield. Under salinity stress, biological yield per plant had the highest positive direct effect on grain yield (0.76) and was followed by harvest index (0.65) (Table 2).

Table 2. Direct (diagonal) and indirect (off-diagonal) effects of different traits in rice on grain yield in control (EC_{iw} ~ 1.2 dS/m) and saline (EC_{iw} ~ 10 dS/m) environments

Characters	Environ-ments	Plant height (cm)	Total tillers per plant	Productive tillers per plant	Panicle lenght (cm)	Biological yield per plant (g)	Harvest index (%)	Genotypic correlation
Plant height (cm)	Normal	**0.03**	0.018	0.02	0.018	0.017	0.007	0.48**
	Saline	**–0.04**	0.003	–0.005	–0.015	–0.01	–0.001	0.25**
Total tillers per plant	Normal	–0.07	**–0.01**	–0.009	–0.004	–0.004	–0.002	0.26**
	Saline	0.007	**–0.07**	–0.04	–0.005	–0.019	–0.003	0.14*
Productive tillers per plant	Normal	–0.10	–0.15	**–0.16**	–0.07	–0.072	–0.028	0.25**
	Saline	–0.01	–0.02	**–0.03**	–0.005	–0.001	–0.006	0.07
Panicle length (cm)	Normal	0.02	0.01	0.01	**0.02**	0.005	0.018	0.66**
	Saline	0.01	0.001	0.002	**0.01**	–0.002	0.001	0.17*
Biological yield per plant (g)	Normal	0.36	0.27	0.26	0.14	**0.57**	0.14	0.69**
	Saline	0.27	0.12	0.017	–0.08	**0.76**	0.03	0.76**
Harvest index (%)	Normal	0.18	0.12	0.12	0.55	0.17	**0.72**	0.85**
	Saline	0.02	0.03	0.13	0.26	0.02	**0.65**	0.67**

Residual effects under normal and saline environments are 0.11 and 0.08, respectively.
* significant at $p = 0.05$; ** significant at $p = 0.01$

Stress indices

The stress susceptibility index (SSI) values for grain yield ranged from 0.35 (HKR 127) to 1.55 (TR-2000-008), whereas indices of other genotypes with higher grain yield fell in between these values under high salinity (Table 3). The association between the SSI and grain yield in salinity revealed negative correlation (r = –0.43). The lower values of SSI indicated lower differences in yield across stress and normal environments and hence more stability and indicate genotypes performing well under stress with sufficient plasticity to respond to the potential environment.

The stress tolerance index (STI) values for grain yield ranged from 0.07 (PR 118) to 1.09 (HKR 120), with the genotypes HKR 120, HKR 47 and CSR-RIL-197 exhibiting higher values of stress tolerance index (Table 3). The association between the STI and grain yield under salinity revealed a strong positive correlation (r = 0.81**) thus indicating better utility of STI in identifying higher yielding genotypes across environments and prediction of stress tolerance as compared to SSI (r = –0.43**) in these materials. Overall results indicate better utility of STI in identifying higher yielding genotypes across environments and prediction of stress tolerance in such materials as compared to SSI.

Table 3. Salinity stress susceptibility (SSI) and salinity stress tolerance (STI) indices for yield and its component traits in rice genotypes

S. No.	Genotypes	SSI				STI			
		Plant height	Prod. tillers	Biolog. yield	Grain yield	Plant height	Prod. tillers	Biolog. yield	Grain yield
1	IR77674-3B-8-1-3-13-2-AJY2	1.12	0.90	1.42	1.31	0.63	0.83	0.43	0.61
2	IR77674-3B-8-1-3-13-4-AJY2	0.89	1.20	1.06	1.12	0.66	0.66	0.26	0.44
3	IR77674-3B-8-1-3-14-2-AJY3	0.93	0.71	1.00	1.11	0.63	0.68	0.32	0.60
4	IR77674-3B-8-1-3-14-2-AJY4	0.87	0.74	1.15	1.22	0.72	0.87	0.26	0.43
5	IR78806-B-B-16-1-2-2-AJY1	0.96	1.12	0.87	0.74	0.70	0.66	0.36	0.48
6	IR60997-16-2-3-2-2R	0.73	0.47	0.95	1.00	0.68	0.74	0.44	0.66
7	BCW 56	1.49	1.57	1.30	1.38	1.01	0.52	1.05	0.59
8	CSR-RIL(CSR 27/MI 48)-50	1.04	0.59	0.61	0.48	0.70	0.83	0.43	0.53
9	CSR-RIL(CSR 27/MI 48)-197	1.01	0.57	0.80	0.71	0.81	0.77	0.60	0.79
10	CSR-RIL(CSR 27/MI 48)-75	0.98	1.63	0.47	0.61	0.77	0.73	0.46	0.51
11	CSR-RIL(CSR 27/MI 48)-192	0.33	1.29	0.64	0.37	0.99	0.84	0.29	0.25
12	CSR-RIL(CSR 27/MI 48)-169	1.13	0.71	1.14	1.04	0.79	0.79	0.71	0.64
13	CSR-RIL(CSR 27/MI 48)-170	1.28	0.83	0.44	0.92	0.59	0.74	0.27	0.19
14	TR-2000-008	1.43	1.77	1.60	1.55	1.31	1.04	0.64	0.17
15	NDR-359	1.26	0.86	0.50	0.80	0.75	0.56	0.31	0.24
16	PR 113	0.85	0.96	0.97	1.06	0.64	0.71	0.24	0.09
17	PR 114	0.66	1.13	0.96	0.68	0.62	0.70	0.36	0.16
18	PR 115	1.11	0.74	0.94	0.56	0.55	0.68	0.35	0.24
19	PR 116	0.65	0.86	1.01	0.78	0.68	0.57	0.49	0.28
20	PR 118	0.29	0.96	0.71	0.87	0.59	0.62	0.29	0.07
21	PAU 201	0.83	0.77	0.94	0.69	0.57	0.71	0.36	0.27
22	PR 120	1.27	1.33	0.95	0.96	0.60	0.68	0.53	0.35
23	PUSA 44	1.12	0.90	1.34	1.24	0.55	0.64	0.27	0.07
24	HKR 46	1.21	0.80	0.82	0.52	0.60	0.61	0.37	0.26
25	HKR 47	1.24	0.51	0.81	1.09	0.74	0.71	0.79	0.84
26	HKR 120	0.68	0.90	1.04	0.95	0.76	0.61	1.00	1.09
27	HKR 127	1.18	0.55	0.68	0.35	0.73	0.62	0.57	0.48
28	CSR 10	1.18	0.83	0.59	0.45	0.53	0.91	0.31	0.15
29	CSR 13	1.09	0.78	0.95	0.67	0.66	0.79	0.56	0.27
30	CSR 23	0.51	1.23	0.96	0.54	0.98	1.05	0.76	0.56
31	CSR 27	1.07	1.30	1.08	0.59	0.83	0.54	0.52	0.58
32	CSR 36	1.04	0.95	0.93	0.50	0.78	0.81	0.96	0.63
33	MI 48	1.31	1.18	0.83	0.80	0.74	0.69	0.42	0.42
34	VSR 156	0.78	1.53	1.39	1.49	1.43	0.67	0.53	0.35
	Mean	0.99	0.98	0.94	0.92	0.75	0.72	0.49	0.43
	Range	0.29–1.49	0.47–1.77	0.44–1.60	0.35–1.55	0.53–1.43	0.52–1.05	0.24–1.05	0.07–1.09

Discussion

Effect of salinity on grain yield and yield attributing traits

Grain yield per plant was the trait most affected by salinity. Reduction in grain yield and its component traits occurs through osmotic effects reducing the ability of plants to take up water and causing reduced growth. These results are in agreement with the earlier reported findings of Mohammadi-Nejad et al. (2008), Yasseen et al. (2010), and Joseph and Jini (2010). In rice, plant height, total number of tillers, panicle length, grain weight per panicle, 1000-seed weight and quantity of grains decreased progressively with increase in salinity levels (Abdullah et al. 2001). Therefore, identification of plant genotypes with tolerance to salt and incorporation of desirable traits into economically useful crop plants will help overcome the effects of salinity on crop productivity. The phenotypic coefficient of variation (PCV) for all the traits indicated influence of environment on the manifestation of these traits. High magnitude (>15%) of genotypic as well as phenotypic coefficients of variation were observed for biological yield per plant, grain yield per plant and harvest index under both the normal and saline environments. Higher estimates of genetic advance (>25%) and heritability were observed for harvest index and biological yield under normal and saline environments. This suggests that the evaluated materials might provide higher response to selection for these traits exhibiting higher heritability along with higher genetic advance owing to their high transmissibility and variability.

Correlation and path analysis

The grain yield or economic yield in almost all crops is referred to as a complex character resulting from multiplicative interactions of several other characters termed as yield contributing components. Thus genetic architecture of grain yield in rice as well as other crops is based on the balance or overall net effect produced by various yield components directly or indirectly by interacting with one another. Therefore, identification of important yield components and information about their associations is very useful for developing efficient breeding strategies for evolving high yielding varieties. Grain yield per plant showed positive significant associations with plant height, total tillers per plant, productive tillers, panicle length, and biological yield and harvest index in normal environment. Under salinity stress, grain yield showed positive significant associations with biological yield and harvest index. These results clearly indicate that selection of genotype under normal and saline conditions should be entirely different. These results are in agreement with the findings of Raju et al. (2003), Ganapathy et al. (2006), Sabesan et al. (2009) and Chakraborty et al. (2010) in rice. The lower estimates of phenotypic correlation coefficient indicate that the relations were affected by environment at the phenotypic level and are in conformity of the findings of Jayasudha and Sharma (2010) and Anbanandan et al. (2009). The differential responses exhibited by the traits under salinity stress might be due to the complexity of the traits under stress. As grain yield is a complex trait governed by many traits with large environmental influence which along with salinity tolerance also being a complex trait governed by multi genes makes improvement of yield under salinity stress conditions a tough challenge for scientists.

A positive association between two traits warrants simultaneous improvement of both the traits while restricting selection to any one of the associated traits. Relative importance of the attributes should therefore be decided on the basis of higher correlation of the trait with grain yield. However, the correlation coefficient alone is inadequate to interpret the cause and effect relationships among the traits and ultimately with yield. Path analysis technique furnishes a method portioning the correlation coefficients into direct and indirect effects providing information on the actual contribution of a trait on the yield. Direct positive effects of these traits on grain yield indicate their importance in determining complex characters and, therefore, should be accorded priority while making selections for improvements in grain yield. Panicle length exerted low but positive direct effect on grain yield, while plant height, total tillers per plant and productive tillers per plant exhibited negative direct effects on grain yield (Sajjad 1990).

Stress indices

Lower values of SSI indicate lower differences in yield across stress and normal environments and hence more stability thereby indicating better performing genotypes under stress and also possessing sufficient plasticity to respond to the potential environment. This suggested that the genotypes developed for salinity tolerance could exhibit higher adaptability and suitability, particularly under saline conditions. Porch (2006) has reported that several genotypes of common beans were superior for heat tolerance based on the stress indices and consistency of their reactions across environments. White and Singh (1991) reported low yield potential and low SSI scores of drought stress in bean indicated that selection for low SSI could identify stress tolerant lines but with low yield potential under non-stress conditions.

Association between the STI and grain yield in salinity revealed a strong positive correlation thus indicating better utility of STI in identifying higher yielding genotypes across environments and prediction of stress tolerance as compared to SSI in these materials. Overall results indicate better utility of STI in identifying higher yielding genotypes across environments and prediction of stress tolerance as compared to SSI in such materials. Genotypes experiencing minimum yield reduction under stress compared to normal conditions are distinguished by stress susceptibility index (Fischer and Maurer 1978). Lower values of stress susceptibility index (SSI) indicated lower differences in yield across stress and normal environments and hence more stability. This indicates genotypes performing well under stress and also have sufficient plasticity to respond to the potential environment. On the other hand, the higher values of stress tolerance index (STI) indicate superiority of genotypes having higher yield potential and stress tolerance as well (Fernandez 1993), thus making it a more desirable and helpful criterion in identifying and developing genotypes having both higher yield and stress tolerance.

The genotypes CSR-RIL-197, CSR 36, HKR 127 and HKR 120 showed better performance in normal and stress environments and could be useful in increasing productivity under saline stress environments. Correlations among grain yield and its contributing traits, direct and indirect effects of different traits on yield are useful in identification of the trait(s) strongly associated with yield under normal and salinity stress. The traits harvest index and biological yield, which are associated with yield emerged as the ideal traits for improvement through selection under saline stress environments and could be used to increase rice productivity under saline stress environments. The negative and significant association between the SSI and grain yield; positive and significant association between the STI and grain yield at salinity could be utilized by rice breeders in identifying higher yielding genotypes having salt tolerance and sensitivity. The stress susceptible and tolerance indices suggest that genotypes developed for salinity tolerance could exhibit higher tolerance, adaptability and suitability.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at http://www.akademiai.com/content/120427/

Electronic Supplementary *Table S1*. List of 34 rice genotypes and their pedigree/geographical location

Electronic Supplementary *Table S2*. Pooled analysis of variance (ANOVA) for grain yield and its attributing traits in control and saline (EC_{iw} ~ 10 dS/m) environments in rice

Electronic Supplementary *Table S3*. Range, mean, coefficient of variation, heritability and genetic advance for grain yield and other attributes in rice in control and saline conditions